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Death and Transfiguration of a Problem[1]

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"Systems attract those who do not succeed in capturing truth in its totality and who want to catch it by the tail. The system is like the tail of the truth but truth itself is like a lizard: it leaves its tail in your hands and runs away knowing that rapidly it will grow a new tail."

Ivan Turgueniev
in a letter to Leo Tolstoi, January, 1857

INTRODUCTION

"The real seriousness," writes Kierkegaard—a name that the Dane pronounces as it should be, the French exactly as it is written, and the American God knows how—"the real seriousness begins only when a man possessing the right experience sees himself constrained by a dominating power to undertake a work in opposition to his tendencies." To write and to deliver lectures is certainly against my natural tendency; I have been constrained. The situation is therefore serious. However, a part of the endeavor has been interesting, namely the composition of the title.

In his *Introduction to Scientific Research*, Bright Wilson (2) states that a title should be selected with care, that it should provide as much information as possible about the nature of the paper, that it should not claim too much, and that its wording should be carefully studied. I have done my best to conform to these excellent principles. As a result of my efforts, the title of the lecture might seem obscure. In fact, according to my own opinion, a title demands a part of mystery or, better, should be a gateway open to a mystery. This one is as secret as one can wish for. Maybe some of you would prefer more light. The light will come, sooner or later.

At this point, let me confess that I am rather worried, for the title, because of the upshot of its secret nature, could only be the best part of the show. In view of their relative length, a bad title and a good lecture would certainly do better. But the lecture lasts only 1 hr, whereas you have been exposed to the title for many months. Whatever the case may be, today, now, at last, you have the right to learn its meaning. Yet, since the waiting has been so long I am quite sure that you are willing to sacrifice a few more minutes of your intellectual comfort for the sake of the suspense; also to allow the lecturer to act in conformity with his own practice, a practice which can be equated to a tradition if it is admitted that an aged scientist is answerable to tradition.

I shall therefore produce some irrelevant remarks. Many times I have spent many months in this country where I have many friends. I almost feel as if I were an American microbiologist. As can easily be judged by the truly un-American title of the lecture, I am not. Before this misbehavior, the American Society for Microbiology had promoted me from ordinary to honorary membership, a rare distinction which I received with the greatest pleasure. This is a unique opportunity to express publicly my

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gratitude to the Society. To be honest, I have to confess that I never had the courage to attend a general meeting. I was afraid to face a few hundred colleagues at the same time. From the quantitative point of view, reality is much worse.

One of the problems which confront the scientist is competition. According to the golden rule of intellectual hygiene, one should forget competition, and this is impossible here. Otherwise, the meeting up to now seems all right. It is, however, regrettable that some participants propose esoteric titles which constitute questions in themselves, as if there were not enough true questions.

One of them is, of course, the relation of all that with the Navy. The help of this most powerful institution could be an expression of the sailors' solidarity. The Navy knew that I had to navigate on the mysterious sea of secret problems, and it provided its assistance. Whatever the case might be, I would like to express my deep appreciation to the Office of Naval Research, which operates the problem; I mean the lecture.

Maybe some of you would like to know what the title means. Anyhow, I must tell you what I have in mind. Reality, I am afraid, will be far less fascinating than mystery. About a secret title one can dream and discuss endlessly all sorts of exciting hypotheses, whereas truth appears trivial. At all events, a suspense must come to an end. The subject of the lecture, yes, the subject of the lecture, is the problem of the mechanism of action—you have certainly been waiting long enough—it is the problem of the mechanism of action. I should say the intimate mechanism of action; it is the problem of the intimate mechanism of action—I am sure you will be deceived—it is the problem of the intimate mechanism of action of supraoptimal temperatures on viral development. As a matter of fact, I am sorry to say, the true problem is something else or perhaps, maybe, something more.

In an infected organism, the virus modifies the cell it infects. The infected cell modifies the organism. The reaction of the organism modifies the infected cell. The alterations of the infected cell block viral development. The problem of the interrelations and significance of these complex events was discussed 10 years ago in a Squibb lecture and reviewed again a few years later (9). In the past few months, some new data and also some new concepts have come to light and the problems can be posed anew. How does fever act at the cellular and molecular level? Virulent viral mutants are able to overcome the defense mechanisms of the organism. What is the molecular basis of virulence? My ambition is to discuss recent data and hypotheses pertaining to these questions.

A lecture delivered on a solemn occasion like this one should be general, that is, it should consist essentially of irrelevant remarks, discussions, digressions, and philosophical concepts. Yet, it is customary for scientists to produce slides. I remember exceedingly well a lecture composed of 27 slides of sucrose gradients. A few experimental data, nevertheless, sometimes seem useful and I hope you will forgive me if curves will appear on the screen.

TERMINOLOGY

Because we are going to discuss temperature, it is necessary first to agree on terminology. Virologists often speak of "temperature-sensitive" or of "thermosensitive" mutants. One should note first that thermosensitivity can apply either to virions or to viral development. Second, whatever the virus, viral development is always sensitive to temperature. It seems probable that virologists designate by thermosensitive or temperature-sensitive those viruses inhibited in their development above 37 C. As everyone knows, the value of the temperature of the human being is a magic and sacred number. Temperature, of course, always controls viral development. The optimal temperature is the one at which the yield in virions is maximal. It is best expressed by the middle value of the optimal zone and its limits; for example, if the optimal zone extends from 33 to 36 C, the optimal zone of temperature is defined by the formula 34.5 $\pm$ 1.5 C. Above and below the optimal zone, the yield is decreased. The *rt* (*r* for reproduction as affected by *t* for temperature) is the temperature at which viral yield is decreased by 94%. One distinguishes an rt^+ for infraoptimal temperatures and an rt^- for supraoptimal temperatures (6*a*). The "temperature formula" of a virus includes the optimal zone, the rt^+ and the rt^-. For example: rt^+ = 30.5 C; optimum = 34.5 $\pm$ 1.5 C; and rt^- = 38.5 C.

The first question is why is viral development blocked at supraoptimal temperatures? It is generally admitted that at these temperatures the synthesis of viral ribonucleic acid (RNA) is blocked, and it is true that a synthesis of viral RNA has not, up to now, been detected at supraoptimal temperatures. If caution is always needed in the interpretation of what is seen, more caution is necessary when one sees nothing. This remark will be illustrated later.

VIRAL RNA SYNTHESIS AT SUPRA-OPTIMAL TEMPERATURES IN A STRAIN rt⁻ 38.5 C

A strain of poliovirus I whose *rt*⁻ is 38.5 C shows no production of virions at all at 40 C. If, however, such a strain is maintained after infection for 2 hr at 40 C and then shifted to 36 C, the production of virions becomes detectable after 5 min and the curve extrapolates at 2 hr (10, 12, 17).

This means that something has taken place at 40 C. From what is known of the kinetics of poliovirus development, it can be stated that viral RNA and capsidal protein are produced at 40 C. Is this conclusion correct?

The synthesis of viral RNA as a function of temperature was measured in infected KB cells. The cells were treated with actinomycin at the time of infection. Under these conditions, the synthesis of cellular RNA after 90 min reaches a very low level—so low that it can be neglected.

Tritiated uridine is added at 90 min; the RNA is extracted and the radioactivity is estimated. The synthesis of viral RNA as a function of temperature is shown in Fig. 2. The *rt*⁻ of the strain is 38.5 C; its optimum is 36 C. At 36 C, one observes a linear synthesis of RNA which lasts around 4 hr. Then the rate of synthesis decreases, and from 4.5 hr on the amount of RNA decreases. This phenomenon, which is not always observed at optimal temperatures, does not seem to have attracted much attention. It will be commented upon later. The higher the temperature, the smaller the synthesis of RNA. At 39.5 C, the production of virions is less than 1% of the maximum, and the synthesis of RNA is hardly measurable.

It is known that at optimal temperatures the synthesis of poliovirus RNA is exponential for 3 hr and then becomes linear. In the experiment corresponding to Fig. 3, the synthesis of viral

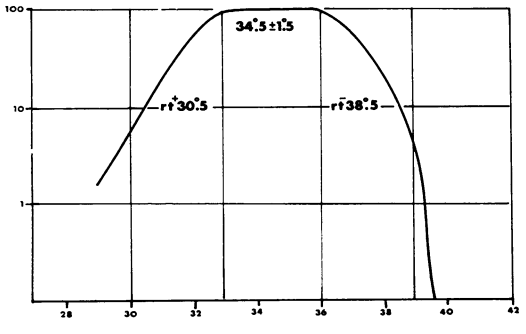

FIG. 1. *Development of a strain of poliovirus as affected by temperature. The virions are in ordinates.*

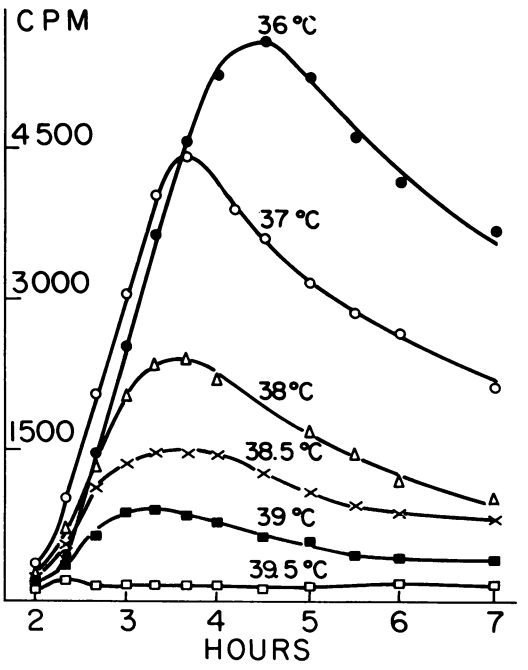

FIG. 2. *Viral RNA synthesis as a function of temperature. Strain of rt⁻ 38.5 C. In all the experiments, unless stated differently, actinomycin was added at time zero (immediately after infection), and tritiated uridine was added at 90 min. Flasks at various temperatures from time zero. (Data are from Fiszman, Bucchini, Girard, and Lwoff, unpublished.)*

RNA has been measured at 36 and at 39.5 C in a strain of *rt* 38.5 C. Both 35*S* RNA and double-stranded RNA have been estimated. Tritiated uridine has been added, as usual, at 90 min. At 2 hr, the level of both ribonucleic acids is the same at 36 and at 39.5 C. This means that viral RNA has been synthesized at equal rates at optimal and supraoptimal temperatures. Between 2 and 3 hr, the synthesis of viral RNA continues exponentially, although the rate is smaller at 39.5 that at 36 C. This applies to single-stranded RNA as well as to double-stranded RNA. Thus, viral RNA is synthesized normally during 2 hr at supraoptimal temperatures. This is confirmed by the following experiment (Fig. 4).

The control was maintained at 36 C; six flasks were kept at 39.5 C and shifted at intervals at 36 C. If the shift took place at 2 hr, the synthesis of RNA was practically identical to that of the control kept at 36 C from time zero. This is additional proof that the synthesis of RNA proceeded normally for 2 hr at 39.5 C and that it was inhibited only later. The state-

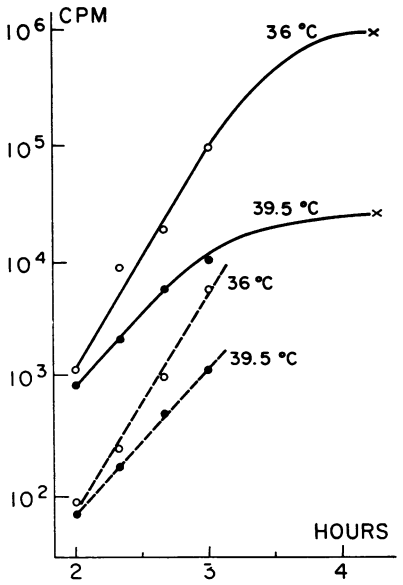

FIG. 3. *Synthesis of viral RNA at optimal and supraoptimal temperatures. Hela cells were infected with poliovirus (rt⁻ 38.5 C). Flasks were maintained either at 36 or at 39.5 C. Upper part: single-stranded viral RNA; lower part: double-stranded RNA (Fiszman, Bucchini, Girard, and Lwoff, unpublished data).*

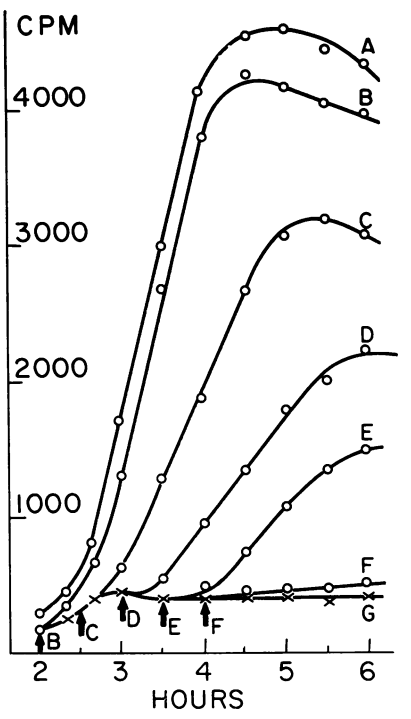

FIG. 4. *Synthesis of viral RNA as a function of the duration of the development at 39.5 C. Poliovirus rt 38.5 C. (A), 36 C throughout; (B to F), 39.5 C at time zero, and transferred at 36 C, respectively, at 2, 2.5, 3, 3.5, and 4 hr; (G), 39.5 C throughout (Fiszman, Bucchini, Girard, and Lwoff, unpublished data).*

ment that supraoptimal temperatures block the synthesis of viral RNA is valid, therefore, only if one arbitrarily neglects the first 2 hr of the life cycle. If the infected cells are kept at 39.5 C for more than 2 hr, the subsequent synthesis of RNA at 36 C is severely upset. The rate decreases as a function of time, and after 4 hr at 39.5 C the synthesis of viral RNA is not resumed. Something has been altered. What?

The following experiment should provide an answer. Two control flasks were kept, one at 36 C, one at 39.5 C throughout 6 hr. The others stayed at 36 C first (Fig. 5) and were transferred to 39.5 C at intervals. (The *rt* of the strain was 38.5 C.) The control showed a typical curve: first, from 2 to 3 hr, the end of the exponential rise of viral RNA; second, a linear phase standing for around 3 hr, which corresponds to the phase of virion morphogenesis.

At 39.5 C, the synthesis of RNA is negligible. The transfer from 36 to 39.5 C is followed by a destruction of viral RNA. The later the transfer, the quicker it starts. More important, the later the transfer the more rapid the destruction. The shift at 4 hr or later induces an almost immediate degradation. Thus, supraoptimal temperatures induce the synthesis, unmasking, or activation of a nuclease. The phenomenon had to be analyzed.

DEGRADATION OF VIRAL RNA DOES NOT REQUIRE PROTEIN SYNTHESIS

It is known that the synthesis of RNA stops rapidly when the protein synthesis is blocked. This is due partly to the fact that the half-life of the replicase is short. If cycloheximide, which blocks protein synthesis, is added to infected cells the synthesis of viral RNA is stopped within 20 min at 36 C (Fig. 6).

If cycloheximide is added just before the transfer at 39.5 C, the viral RNA is degraded "normally." The same degradation is observed if cycloheximide is added 30 min before the transfer. This means that protein synthesis is not necessary for the onset of nuclease activity; at 36 C, the nuclease is present in the infected cells. The shift to higher temperatures only reveals its presence.

Thus, when infected cells are transferred at

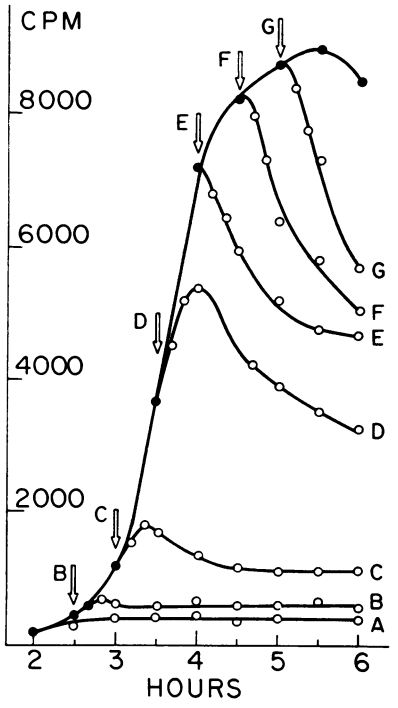

FIG. 5. *Degradation of viral RNA after transfer at supraoptimal temperature. Poliovirus rt 38.5 C. (A) Two controls, one at 36 C (●), the other at 39.5 C (○) throughout; (B to G) infected cells at 36 C from time zero transferred at 39.5 C at the time indicated by arrows (5).*

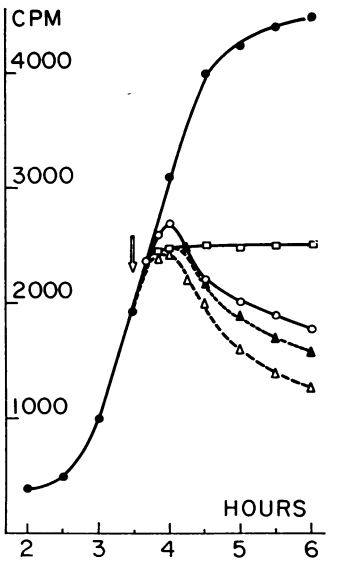

FIG. 6. *Effect of the inhibition of protein synthesis on the degradation of RNA (5). Poliovirus rt 38.5 C. Symbols: ●, 36 C throughout, no addition; □, 36 C throughout, cycloheximid (100 μg/ml) added at 3.5 hr; △, 36 C from time zero, and transferred to 39.5 C at 3.5 hr; ○, 36 C from time zero, cycloheximid added and transferred to 39.5 C at 3.5 hr; ▲, 36 C from time zero, cycloheximid added at 3.5 hr and transferred to 39.5 C at 4 hr.*

supraoptimal temperatures, the rate of RNA synthesis first decreases, then stops, and RNA is finally degraded. How does the replicase behave during these three phases?

REPLICASE ACTIVITY AT OPTIMAL AND SUPRAOPTIMAL TEMPERATURES

The infected cells (*rt*⁻ 38.5 C) were incubated at 36 C (Fig. 7). The transfer of some of the flasks was made at 3 hr. In Fig. 7A, tritiated uridine was added at 90 min. Figures 7B and 7C correspond to pulses of 10 min duration at various intervals. At 36 C, the rate of synthesis of RNA first was constant then decreased after 4.5 hr. At 39.5 C, the rate of synthesis started decreasing immediately and came to a standstill. Figure 7C corresponds to the cumulation of the pulses. It is clear that after the transfer at 39.5 C a synthesis of viral RNA takes place for around 40 min.

The same type of experiment has been repeated with a strain of *rt* 40.8 C. Here also, the synthesis of viral RNA continued at 39.5 C, but was detectable for 80 min instead of 40 (Fig 8). Thus, the replicase of the *rt* 40.8 C strain is less sensitive to supraoptimal temperatures than that of the 38.5 C strain. If there is any logic in a virus, this is logical. Later on, other experimental data will confirm this conclusion.

The fact that a synthesis of RNA is not detectable after 1 hr could mean that the destruction of the RNA exactly compensates its synthesis. This is unlikely. The hypothesis according to which supraoptimal temperatures block the activity of the replicase is more attractive. Can such an inhibition be detected in vitro? Up to now, the activity of the purified poliovirus replicase in vitro was low and lasted only for 20 or 30 min. Marc Girard (Virology, *in press*) succeeded in obtaining an active and relatively stable enzyme which works for more than 2 hr. As can be seen, at 40 C the activity came to a standstill but RNA was not degraded (Fig. 9).

SOMETHING IS DESTROYED AT SUPRAOPTIMAL TEMPERATURES

A mere inhibition of the replicase activity, however, would be reversible, whereas the action

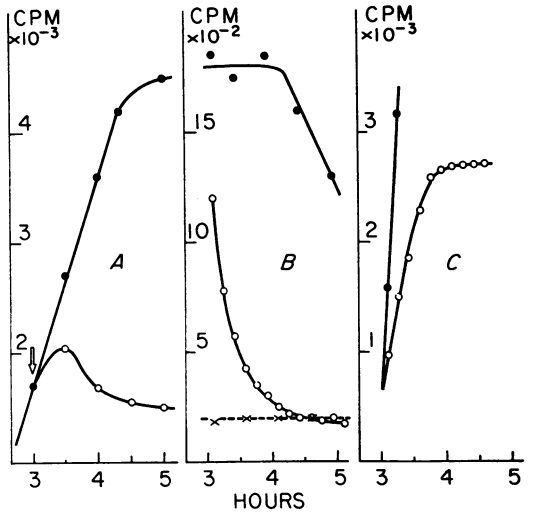

FIG. 7. *Activity of the replicase after transfer of infected cells at supraoptimal temperature. Symbols: ●, 36 C throughout; ○, 36 C from time zero and transferred to 39.5 C at 3 hr; ×, 39.5 C throughout. (A) Tritiated uridine was added at 90 min. (B) Pulses (10-min) of tritiated uridine. (C) Cumulative curve (Fiszman, Bucchini, Girard, and Lwoff, unpublished data.)*

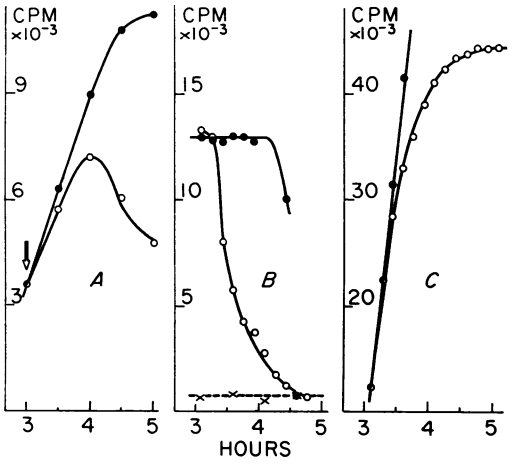

FIG. 8. *Activity of the replicase after transfer of infected cells at supraoptimal temperature. Same experiment as in Fig. 7, but strain of rt 40.8 C.*

of supraoptimal temperatures becomes irreversible after a while. This statement is based first on the study of the production of virions and then on the study of the RNA synthesis, which gives similar results (Fig. 10). Infected cells were kept at 36 C. One control flask was left at 36 C. Others were transferred to 39.5 C

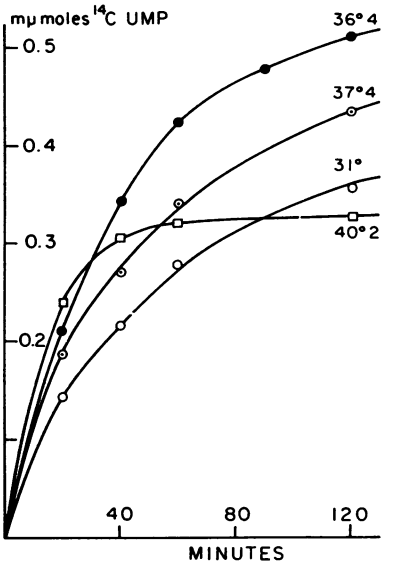

FIG. 9. *Activity of the replicase in vitro at optimal and supraoptimal temperatures. Incorporation of ¹⁴C-uridine monophosphate by the purified replicase (M. Girard, J. Virol., in press).*

after 2.5 hr and, at 10-min intervals, were retransferred at 36 C. After 10 min at 39.5 C, the RNA synthesis at 36 C was hardly modified. However, the longer the flask stayed at 39.5 C, the slower was the rate of synthesis at 36 C and the lower was the total synthesis. After 60 min at 39.5 C, the synthesis of RNA was not resumed. Therefore, at 39.5 C something was destroyed which controls RNA synthesis and its rate.

It is possible that during the linear phase the rate of RNA synthesis is controlled by the number of templates, that is, in the last analysis by the number of minus strands. The decreasing rate of synthesis could express the destruction of the free minus strands by the nuclease.

WHERE DOES THE RIBONUCLEASE COME FROM?

Be that as it may, with the viral RNA being destroyed at supraoptimal temperatures, a ribonuclease is necessarily involved. Where does the nuclease come from? The deoxyribonucleic acid (DNA) polymerase exhibits, under certain circumstances, a nuclease activity. One hypothesis was pleasant: the nuclease results from an allosteric transition of the replicase. The supraoptimal temperatures would shift the equilibrium replicase-nuclease towards the right. As you might remember, a destruction of viral

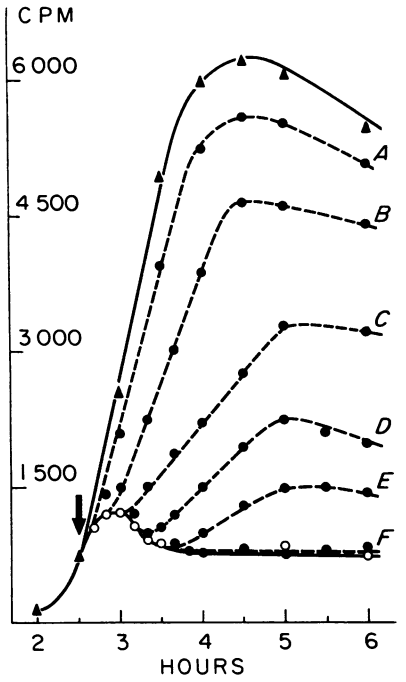

FIG. 10. *Synthesis of RNA as a function of the duration of the development at 39.5 C. Poliovirus rt 38.5 C. Full lines represent two controls, 36 and 39.5 C. Flasks A–F were at 36 C from time zero, shifted to 39.5 C at 2.5 hr, and shifted back to 36 C after 10, 20, 30, 40, 50, and 60 min, respectively (Fiszman, Bucchini, Girard, and Lwoff, unpublished data).*

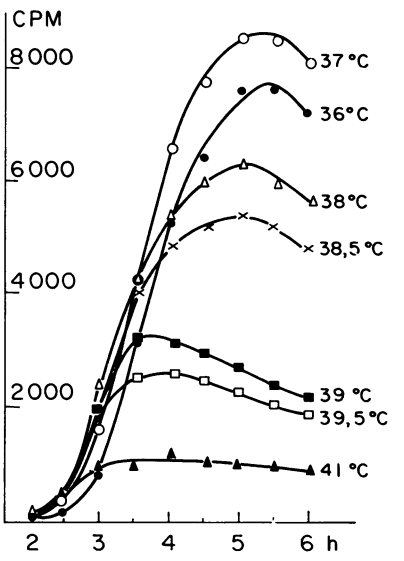

FIG. 11. *Synthesis of RNA by a strain of rt⁻ 40.8 C. The infected cells are kept at various temperatures from time zero (Fiszman, Bucchini, Firard, and Lwoff, unpublished data).*

RNA sometimes takes place at optimal temperatures towards the end of the cycle. This is not in contradiction with the hypothesis, for allosteric enzymes are very sensitive to all sorts of factors. The replicase of the poliovirus is highly sensitive. So the cellular alterations occurring as the result of viral development could, by themselves, be responsible for the transition replicase → nuclease. Supraoptimal temperatures would simply favor the transition. Reality can afford to be uninteresting—hypotheses cannot. Besides being exciting, our hypothesis had one advantage: it offered a clue to the riddle of the origin of the RNA viruses. RNA is not supposed to be replicated in a normal cell. A normal cell does not contain replicases, only ribonucleases. Let us now imagine that the structural gene of a ribonuclease undergoes a mutation which shifts the balance replicase → nuclease towards the left; the ribonuclease will act as a replicase and replicate its own messenger. This is necessarily the first step in the phylogeny of an RNA virus. Of course, this primitive virus is reduced to its genetic material and a number of viral functions are missing, but the production of a capsid can be considered as a relatively simple technical problem. The important phenomenon, here as anywhere else, is the reproduction of the genetic material.

Montagnier recently discovered a double-stranded RNA in "normal" animal cells, normal meaning noninfected by a virus (16). For various reasons, Montagnier favors the hypothesis according to which the double-stranded RNA results from the replication of a messenger RNA. If the replicase → nuclease transition hypothesis corresponds to reality, this would mean that some animal cells are trying to generate RNA viruses, although they have not yet succeeded. This is exciting. But a hypothesis is useful only if it can be disproven. Therefore, the replicase → nuclease transition hypothesis was submitted to experimental tests.

BEHAVIOR OF A STRAIN OF rt⁻ 40.8 C

We know that at 38 C, or even at 37 C, the development of the wild strain of *rt* 38.5 C is inhibited by a nuclease. What happens at these temperatures to strains of higher *rt*? These are easy to obtain by selecting mutants able to grow at high temperatures. We have studied, especially, a strain of *rt*⁻ 40.8 C. Its optimum is around 37 C (12).

In one experiment, the infected cells were maintained at various temperatures from the

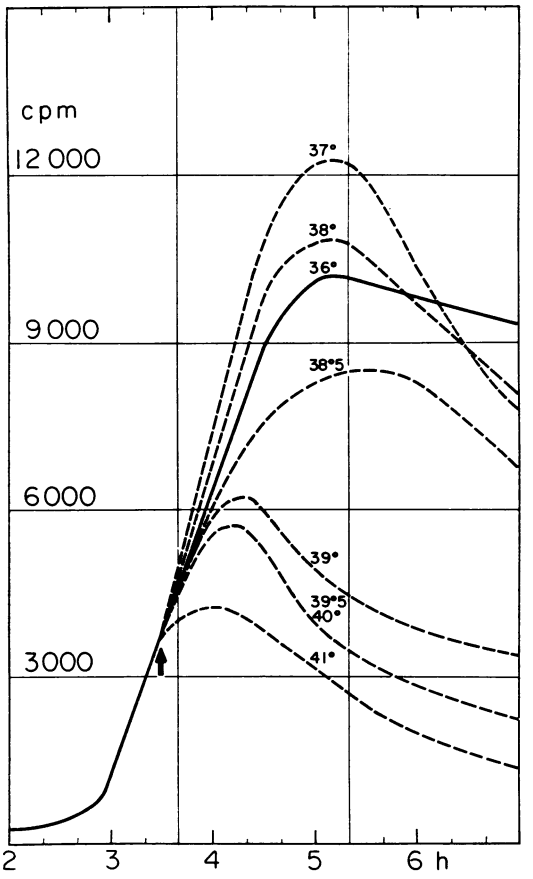

FIG. 12. *RNA synthesis after shift at various temperatures. Poliovirus rt⁻ 40.8 C. After 3.75 hr at 36 C, the infected cells were transferred at various temperatures (Fiszman, Bucchini, Girard and Lwoff, unpublished data).*

time of infection, and the synthesis of viral RNA was measured (Fig. 11). The synthesis of RNA was quicker and more important at 37 than at 36 C; it was lower at 38 C, but the degradation of the RNA took place whatever the temperature. A nuclease was at work at 37 C despite the fact that 37 C is the optimal temperature.

In another experiment, the infected cells were maintained for 3.75 hr at 36 C and either were kept at 36 C or transferred at various temperatures (Fig. 12). The synthesis of viral RNA was more rapid and more important at 37 and 38 C than at 36 C. Whatever the temperature, optimal or not, the RNA was degraded. The higher the temperature, the earlier the degradation.

From all these data, it can be concluded that

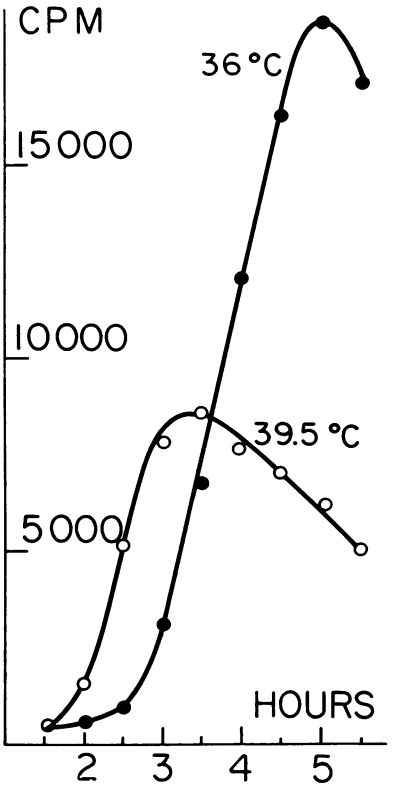

FIG. 13. *Synthesis of RNA at 36 and 39.5 C. Poliovirus rt 40.8 C. The infected cells were maintained, respectively, at 36 and 39.5 C throughout the experiment (Fiszman, Bucchini, Girard, and Lwoff, unpublished data).*

with the "hot" strain of *rt*⁻ 40.8 C, just as with the strain of *rt*⁻ 38.5 C, a nuclease interferes with viral development even at 37 C, which is the optimal temperature. Why, then, is the hot strain able to develop at temperatures above 36 C? Infected cells were maintained at 36 or 39.5 C from the time of infection (Fig. 13). It is clear that at 39.5 C the exponential phase of RNA synthesis was shorter than at 36 C. In many experiments, the rate of synthesis during the linear phase is higher at 39.5 than at 36 C. Thus, when the nuclease activity appears, the viral RNA has reached a relatively high level.

Thus, at supraoptimal temperatures, mutants are selected whose replicase is more active at supraoptimal temperatures than at optimal ones. However, the nuclease activity is present. Therefore, the hypothesis according to which, in strains of high *rt*, the balance replicase → nuclease at supraoptimal temperatures is shifted to the left was not confirmed. This was depressing, but there was still a possibility that the mutants

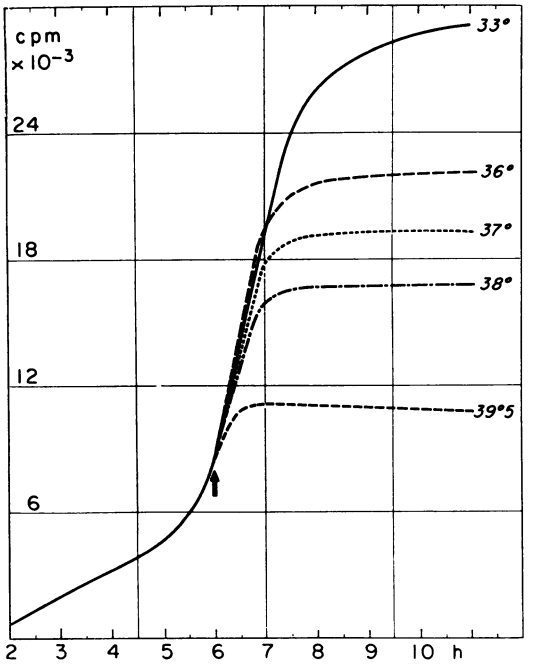

FIG. 14. *Synthesis of viral RNA as a function of temperature. Poliovirus rt⁻ 37.5 C. All of the flasks were maintained at the optimal temperature (33 C) from time zero. Four flasks were shifted at supraoptimal temperatures at 6 hr (Fiszman, Bucchini, Girard, and Lwoff, unpublished data).*

with a low *rt* could behave according to our hopes.

BEHAVIOR OF A STRAIN OF rt 37.5 C

We studied a mutant with an rt⁻ 37.5 C that had been selected by repeated passages at low temperatures (7). Infected cells were grown at 33 C, and the flasks were then transferred at higher temperatures (Fig. 14). (Please note that the transfer was made at 6 hr.) The synthesis of the RNA was stopped; the higher the temperature, the earlier the inhibition. Contrary to expectation, there was no visible nuclease activity. Things seemed more and more depressing until it was decided that the results were highly instructive. Here, supraoptimal temperatures had not triggered a nuclease but had blocked the activity of the replicase.

What happens if the temperature shift is performed later? If the transfer at 39.5 C was made at 6 hr, nothing happened, exactly like the preceding experiment (Fig. 15). The same was true at 7 hr. However, if the infected cells were transferred at 39.5 C after 8 hr, a degradation of the viral RNA did take place.

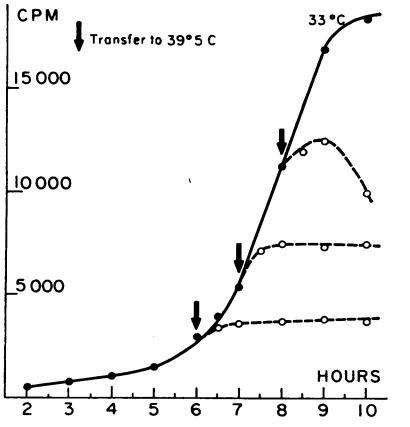

FIG. 15. *RNA synthesis after transfer at supraoptimal temperature. Poliovirus rt⁻ 37.5 C. Infected cells were at optimal temperature (33 C) at time zero. Transfer was made at 39.5 C to 6, 7 and 8 hr (Fiszman, Bucchini, Girard, and Lwoff, unpublished data).*

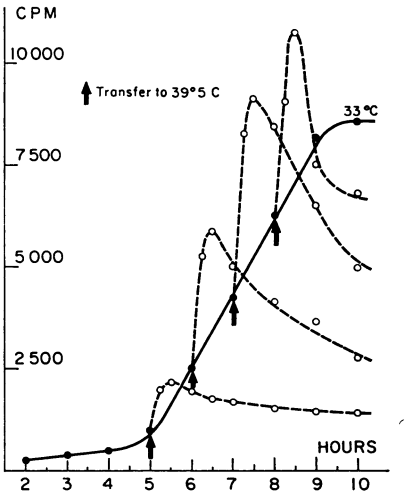

FIG. 16. *Synthesis of RNA after the transfer at 39.5 C following development at infraoptimal temperature. Poliovirus rt⁻ 38.5 C. Infected cells were at infraoptimal temperature (33 C). Transfer was made to 39.5 C after 5, 6, 7, and 8 hr, respectively (Fiszman, Bucchini, Girard, and Lwoff, unpulished data).*

This means that high temperatures do not by themselves induce a nuclease activity. Things happen as if viral development had to reach a certain level before the nuclease activity can be detected. This level is controlled by the genetic constitution of the virus, the multiplicity, the temperature, and the time: a number of parameters. At a low temperature, let us say 33 C, viral development is certainly impaired, and

maybe there is some unbalance between the synthesis of RNA and the synthesis of proteins. Cells infected by a strain of *rt*⁻ 38.5 C were kept at 33 C (Fig. 16). At intervals, flasks were shifted to 39.5 C. The shift was followed by an immediate and very rapid synthesis of viral RNA. Either the template or the replicase had cumulated at 33 C. It is obvious that the replicase works very well at supraoptimal temperatures. The curves show that the degradation started 30 min after the transfer at 39.5 C: the later the shift, the quicker the destruction of viral RNA. In this experiment, again, the activity of the nuclease was related to the importance of viral development.

A digression here is useful. Wentworth and her co-workers (19) described mutants of poliovirus which do not produce virions at 40 C but which are supposed to synthesize infectious RNA normally. Two of these mutants were provided by P. D. Cooper. Marc Fiszman found that they behave exactly as did our strain of *rt* 38.5 C (*unpublished data*). One should not conclude that mutants with the proprieties ascribed to them by Wentworth et al. do not exist, but simply that their reality should be reinvestigated. We have to come back to our problem.

DISCOVERY OF THE LYSOSOME

According to the working hypothesis, the nuclease is an altered replicase. If it were so, the phenomenon should be independent of the phase of the viral cycle, and it is not. Therefore, doubts began to creep into our minds. Yet, allosteric enzymes are sensitive molecules easily affected by the conditions of the environment. As the virus develops, the cell is certainly altered, and you might remember that a nuclease activity is sometimes observed at optimal temperatures towards the end of the cycle. Therefore, the replicase → nuclease conversion theory was not necessarily wrong. The suspicion was there, however, and serious worries about its legitimacy were developing.

You know how things are. A number of interesting molecules, particles, and concepts are buried in scientific papers. They do not exist until one becomes aware of their existence. If we really knew everything we should know, we would be discouraged, depressed, and intellectually sterilized. The scientist often avoids being learned because erudition kills imagination. He has to stay halfway between ignorance and the vague feeling that certain things do exist. It is while in that obscure and fortunate state of mind that I discovered the lysosome. As noted by a philosopher, man is unable not to appropriate to himself what seems so beautifully made *for* him that, against his own will, he regards it made *by* him.

Lysosomes, as will be seen, were so exactly made for me that it is against my will that I claim their discovery. In fact, I am afraid they were discovered by DeDuve (4). Lysosomes, according to DeDuve, are bags of acid hydrolases exhibiting structure-linked latency. They contain, among other enzymes, β-glucuronidase, acid proteases, an acid phosphatase, an acid desoxyribonuclease, and an acid ribonuclease. These enzymes are, of course, synthesized just like other proteins, but their free form is only transitory. They become rapidly enveloped by a membrane, and the result is the lysosome. Lysosomes are polymorphic and also physically and chemically heterogeneous. Their size, internal structure, and function vary. Moreover, they are labile. Their membrane is easily damaged and, as a result, the lysosomal enzymes escape in the cytoplasm. This escape is held responsible for cellular lesions. In any event, lysosomes are involved in multiple ways in cellular injury, tissue regression, and necrosis (4). Cortisone and antihistaminic drugs strengthen the lysosomes.

LYSOSOMES AS AFFECTED BY VIRAL DEVELOPMENT

The development of a virus necessarily produces alterations of the cellular metabolism. Allison and Sandelin (1) showed that during viral development lysosomal enzymes are released in the cytoplasm. This was confirmed and extended, especially by Malucci and Allison (15), Wolff and Bubel (20), Thacore and Wolff (18), Hotham-Inglewski and Ludwig (8), and by Flanagan (6). This is by no means an exhaustive list of authors.

According to Flanagan (6), during the development of the poliovirus there is a decrease of lysosomal enzymes, whereas the cell sap is enriched in acid hydrolases (Fig. 17). Thus, during viral development a substance is produced which damages the lysosomes, and the damage may be responsible for cellular alterations.

It should be added (i) that viral development is neither associated necessarily with the release of lysosomal enzyme nor with visible cellular lesions; (ii) that the release of lysosomal enzymes is not necessarily associated with visible cell damage; and (iii) that cell damage is not necessarily associated with a measurable release of lysosomal enzyme (Table 1).

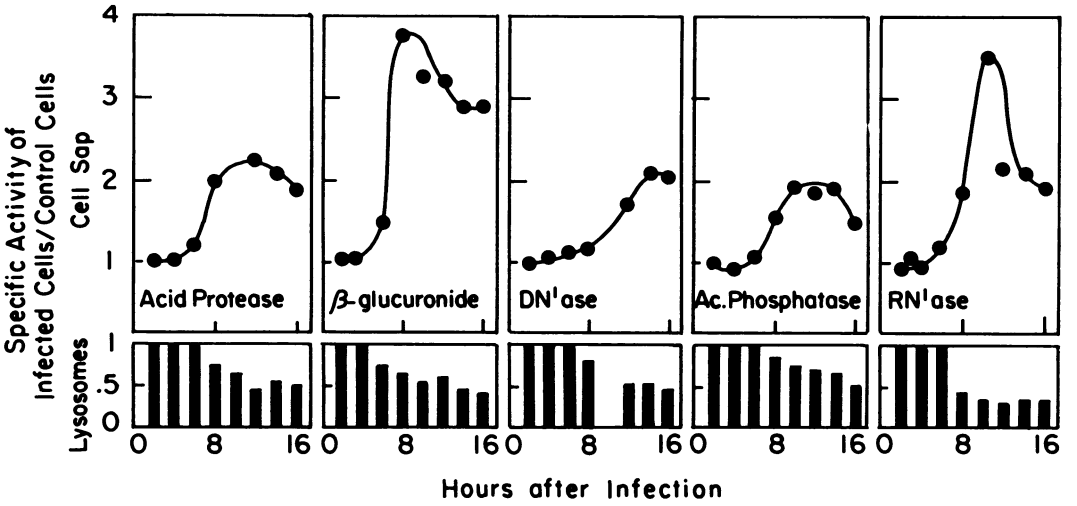

FIG. 17. *Acid hydrolase in the lysosomes and the cell sap of normal and infected cells [from Flanagan (6), with the kind permission of the Journal of Bacteriology].*

TABLE 1. *Release of lysosomal enzymes*[a]

Measurable release of lysosomal enzymes	Visible cellular lesions
+	+
0	0
+	0
0	+

[a] Cellular lesions, in the infected cell, can develop in the absence of measurable release of lysosomal enzymes.

NEW HYPOTHESIS

In view of the data concerning the effects of viral development on lysosomes, a new hypothesis was considered. The appearance of nuclease activity observed at supraoptimal temperatures in poliovirus-infected cells could perhaps be the result of the combined effects on the lysosomes of viral development and of supraoptimal temperatures. Cortisone, as already mentioned, is known to protect the lysosomes. According to Hotham-Iglewski and Ludwig (8), in cells infected with mengovirus, cortisone reduces the release of ribosomal enzymes in the cytoplasm (Fig. 18).

If the action of supraoptimal temperatures is mediated by the lysosomes, one should expect a beneficial effect of cortisone for the virus. Therefore, viral RNA synthesis and viral development were compared at optimal and supraoptimal temperatures in the absence and in the presence of cortisone. Cortisone had no effect whatsoever. This is not surprising, for according to Flanagan (6) the lysosomes of KB cells are not protected by cortisone against the damaging effects produced by poliovirus. Perhaps the lysosomal lesions are too severe.

I would like to recall that a substance is known which modifies the action of temperatures on viral development, namely heavy water. Heavy water decreases the effect of supraoptimal temperatures (3, 13) and increases the effect of infraoptimal temperatures (13). Deuterium oxide probably interferes with the activity of the replicase. In addition, if our concept contains some truth, it should also increase the resistance of the lysosomal membranes to the combined effects of the virus and of the supraoptimal temperatures.

Let us now separate the wheat from the chaff and consider separately the hard fact and the hypotheses.

It is a fact that during a viral infection lysosomes are damaged and lysosomal enzymes are released in the cytoplasm of the infected cell. It is a fact that among these enzymes is a ribonuclease. It is a fact that viral RNA is sometimes destroyed at optimal temperatures towards the end of the life cycle. It is a fact that supraoptimal temperatures trigger a destruction of viral RNA. A ribonuclease is necessarily involved in the hydrolysis. It is a fact that protein synthesis is not necessary for the appearance of ribonuclease activity at supraoptimal temperatures. That the lysosomal ribonuclease released during viral

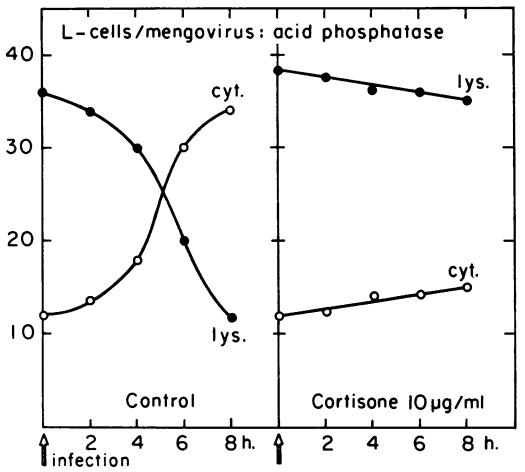

FIG. 18. *Effect of cortisone on the distribution of acid phosphatase between the lysosomes and the cytoplasm. L cells infected with mengovirus (8).*

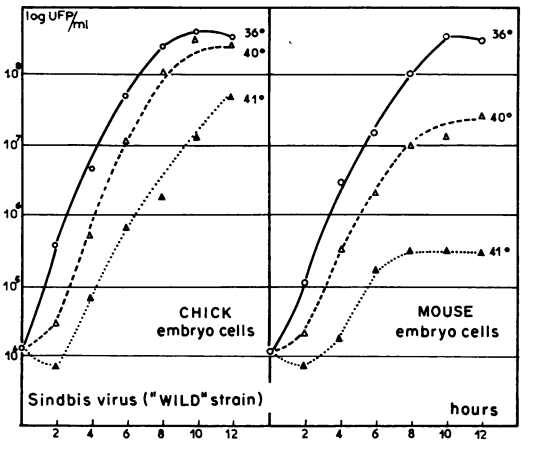

FIG. 19. *Effect of temperature on the development of the Sindbis virus as a function of the host (with the kind permission of Lab and Kirn.*

infection is responsible for the destruction of viral RNA is a hypothesis. That supraoptimal temperatures enhance the virus-induced alteration of the lysosomes is also a hypothesis.

Facts and hypotheses duly weighted, it seems likely that supraoptimal temperatures act through the ribonuclease liberated by the altered lysosomes. If we forget for a while that supraoptimal temperatures inhibit the activity of the replicase, we reach a general hypothesis, according to which the main action of supraoptimal temperatures on viral development is mediated by the cell. If the concept corresponds to the truth, then the effects of supraoptimal temperatures on viral development should depend not only on the genetic constitution of the virus but also on the physiology of the host cell. In some cases, the optimal temperature of the virus corresponds to the normal temperature of the organism from which the cell has been grown. What is supraoptimal for the virus could also be supraoptimal for the cell and the lysosome. If this were the case, the cells and lysosomes of a warm animal like the chicken could be less sensitive to temperatures above 37 C than those of a mouse. Consequently, viral development should be less affected by supraoptimal temperatures in the cells of a chicken than in the cells of a mouse.

Lab and Kirn (8*a*) studied comparatively the development of Sindbis virus in chicken cells and in mouse cells. High temperatures affect the development at considerable extent in mouse cells and much less in chicken cells (Fig. 19). The temperature of the mouse is 38 C, whereas that of the chicken is 41 C. Therefore, for the chicken cells 41 C is physiological. The conclusion is inescapable that the cell takes part in the inhibitory action of supraoptimal temperatures on viral development.

We know that the genetic constitution of the virus controls the activity of the replicase at infraoptimal and supraoptimal temperatures. But the genetic constitution of the virus is only one of the factors which control viral development as affected by temperature. The other is the genetic constitution of the cell, and the action of the cell has to be mediated by some organelle or molecule. The best candidate is the lysosome and its ribonuclease.

Lysosomes are generally considered to be part of the suicidal machinery of the cell, and this might be true. However, the fact that lysosomal enzymes may kill the cell is not in contradiction with their useful role during viral infection. The liberation of lysosomal enzymes, despite the fact that it kills the infected cell, could be an efficient defense mechanism against the virus. The death of a cell is sometimes beneficial for the organism.

FIGHT OF THE ORGANISM AGAINST THE VIRUS

Let me recall briefly how the organism fights against a primary viral infection before antibodies are at work. An organsim is infected by a virus. Leukocytes accumulate around infected cells. As a consequence of the conditions prevailing in the inflammatory zone, the lactic acid accumulates, the CO_2 tension is increased,

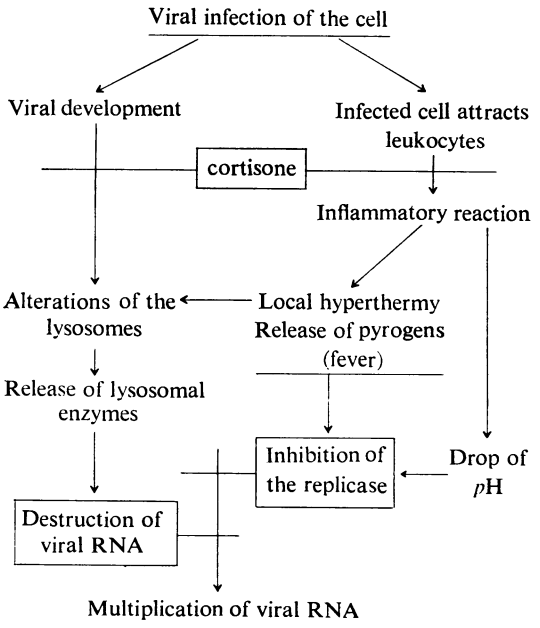

FIG. 20. *Fight of the organism against a viral infection.*

and the *p*H drops to levels incompatible with viral development (Fig. 20). As a consequence of cellular metabolism, the temperature of the inflammatory zone is increased. Moreover, leukocytes release a pyrogenic substance which acts on the central nervous system. Fever is triggered. Temperatures above the normal temperature of the host are supraoptimal for many viral strains; they inhibit viral replicase. Finally, a substance is produced during viral development which alters the lysosomes. Supraoptimal temperatures, in one way or another, increase the lysosomal lesions; lysosomal enzymes are liberated. Among them is a ribonuclease which destroys the viral RNA. Viral development is blocked. If this picture corresponds to reality, lysosomes could be something more than a killing machine; they would play an essential role in the fight of the organism against viruses. It is well known that cortisone increases the severity of viral infection. This is certainly due partly to the decrease of the intensity of the inflammatory reaction. It could also be due, perhaps, to the fact that cortisone, under certain conditions which remain to be ascertained, strengthens the lysosomes and thus prevents their playing their role in the fight against the virus, thus decreasing the beneficial effect of the fever.

Viruses with a high rt^- (that is, able to develop well above 37 C) are more virulent than are those with a low rt^- (that is, those whose optimal temperature is below 37 C). We know today that virulent viruses develop more rapidly than mild ones and that their development at supraoptimal temperatures takes place before the nuclease is at work.

Their replicase is especially active above the normal temperature of the organism. It is, and it must be, for in the light of our actual knowledge a rapid development of the virus at suprapysiological temperatures is a prerequisite for virulence. One could, of course, conceive of viruses owing their low virulence to the fact that they do not alter the lysosomes. Such viruses should certainly be looked for. For the time being, we have a partial knowledge of the meaning of virulence at the molecular level, and what is perhaps more important, we know where to look for further progress.

It was admitted that supraoptimal temperatures inhibit viral development by blocking the synthesis of viral ribonucleic acid. This is a gross expression of a very complex phenomenon. First, during the latent phase the synthesis of viral RNA is perfectly normal at supraoptimal temperatures. Second, it is true that later in the cycle supraoptimal temperatures block the activity of the replicase. However, the main mechanism by which viral multiplication is really stopped is the destruction of viral RNA by a nuclease.

A coherent, unifying concept has been proposed which accounts for numerous data pertaining to infection, temperature, fever, viral development, nucleic acid, lysosomes, and virulence. As coherent as the concept may be, the problem is certainly not solved.

A painting comprises zones of light and zones of darkness which, says Paul Valéry, have to be distributed with art in order to act insidiously on the spectator. The eyes of the spectator are caught by what is limpid, but the zones of chiaroscuro and the interplay of light and shades "exert a secret action, awake forewarnings, questions, enigmas and undefinable beginnings."

I have played with lights and shades. Zones of darkness still persist. To put a brave face on things, the best is probably to decide that shades will generate anxiety, the main driving power of the scientist.

The experiments on which this lecture is based have been performed by Marc Fiszman, Danielle Bucchini, Marc Girard, and myself. Some of the proposed hypotheses or concepts were born as a result of our discussions, and a paper has now been sent to press by the four of

us. It is different from the lecture, which necessarily reflects my own concept of design and my own scientific idiosyncrasy.

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